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(54) Enzyme detergent composition

(57) A detergent composition comprising an alkali cellulase is particularly effective for removing solid, inorganic dirt and enhances the deterging effect of phosphorus free or low phosphorus content detergents. The cellulase may be obtained from *Bacillus* or *Aeromonas* species.

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shape. The alkali cellulase may be incorporated in a composition in the form of any of spray-dried powder, powder-blended powder, tablets or liquid to obtain the detergent composition of the present invention.

The alkali cellulases used in the present invention are those having an optimum pH of 8.0—11.5, preferably 8.1—11.0. Under alkaline conditions, those enzymes exhibit an activity equivalent to that exhibited under acidic or neutral conditions. The alkali cellulases are obtained from cellulase culture liquids of various origins by refining and fractionation according to salting out, precipitation, dialysis or gel permeation method. The alkali cellulases are obtained also by fractionation of crude enzymes or refined enzymes according to electrophoresis or by the heat treatment of the same (for example, at 40—90°C for 0.5 min to 3h).

As particularly preferred alkali cellulases used in the present invention, the following enzymes may be mentioned:

The specific cellulase to be used in the invention is preferably a cellulase produced by a fungus of Bacillus N or a cellulase 212-producing fungus belonging to the genus Aeromonas. The Bacillus N is disclosed in Japanese patent publication No. 28515/75 and available from the Fermentation Research Institute, the Agency of Industrial Science and Technology, located at 1-1-3, Higashi, Tsukuba-Yatabe-machi, Ibaraki, Japan. It has been added to its permanent collection of micro-organisms as deposition numbers, FERM Nos. 1138, 1139, 1140 and 1141. The Aeromonas fungus has been added also to the permanent collection of microorganism in the Fermentation Research Institute, the Agency of Industrial Science and Technology, as FERM No. 2306 and is disclosed in Japanese patent publication (unexamined) No. 76287/75, now published after examination as Japanese patent publication No. 39191/81.

The cellulase to be used in the invention includes a cellulase extracted from the hepatopancreas of a marine mollusc (Dolabella Auricula Solander), which is disclosed in Biochem. J. (1966) *99*, 214—221.

Each of celluloses produced by these fungi is a special cellulase which retains a high activity even under alkaline conditions and has an alkali resistance.

The detergent composition of the present invention is characterized in that this special cellulase is contained as one indispensable ingredient. More particularly, the present invention provides a detergent composition having a prominent washing power to inorganic stains irrelevant to the inherent activity of the cellulase, especially collar contaminations consisting of mixtures of inorganic stains and oils secreted on the skin surface, which change with the lapse of time.

When the detergent composition comprising a special cellulase having a high activity under alkaline conditions and also having an alkali resistance, which is produced by a fungus selected from *Bacillus* N (deposited with FRI deposition numbers of 1138 through 1141) and a cellulase 212-producing fungus belonging to the genus *Aeromonas*, is employed, an excellent washing effect can be obtained over a broad range of the pH value of the washing bath.

This excellent effect overbalances reduction of the washing power due to reduction of the alkali capacity of the builder on reduction of the pH value of the washing bath.

The enzymatic activity of the cellulase that is used in the present invention is determined according to the following method.

50mg of Avicel (for chromatography) or carboxymethyl cellulose (CMC) is suspended in 4ml of a glycine NaCl-NaOH buffer solution (having a pH value of 8,3), and the suspension is preheated at 37°C for 5 minutes and 1ml of an enzyme liquid is added to the suspension. The suspenion is sufficiently mixed and reaction is carried out for 1 hour. After completion of the reaction, the quantity of reduced sugar is determined according to the 3,5-dinitrosalicyclic acid method. More specifically, the liquid reaction mixture is filtered, and 3ml of 3,5-dinitrosalicylic acid is added to 1 ml of the filtrate and the mixture is heated at 100°C for 10 minutes to effect coloration. The mixture is cooled and is then mixed with deionized water so that the total volume is increased to 25ml. The resulting liquid is subjected to colorimetry at a wavelength of 500mμ.

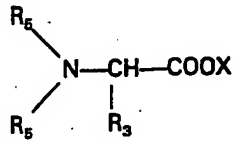
When 1mg of the enzyme as the solid produces reduced sugar in an amount corresponding to 1μ mole of glucose for 1 hour under the above conditions, the enzymatic activity is defined as 1 unit per mg of the solid.

The present invention is attained by combining those alkali cellulases with well-known detergent compositions. As for the alkali cellulase content, it is preferred that the composition contains 0.01—70 wt.%, particularly 0.1—10 wt.%, of an alkali cellulase having an enzymatic activity of at least 0.001 unit/mg solid [1 unit/mg solid forms 10μ mol of glucose from cellulose in one hour at 37°C at pH 8.3]. Also the amount of the alkali cellulase is such that enzymatic activity of alkali cellulase in the bath is preferably 0.1—1000 units/l, more particularly, 1—100 units/l.

Though the detergent composition of the present invention can be used in an unlimited pH range of from acidic to alkaline pH, it is preferred in order to sufficiently exhibit the deterging effect of the alkali cellulase that the deterging bath is alkaline (more particularly, it has pH 7—11).

In the detergent composition of the present invention, components other than cellulase are not particularly limited. For example, the following components may be incorporated in the composition according to their essential properties:

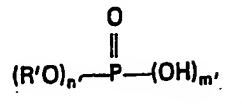
No. 6



wherein R_3, R_5 and X have the same meaning as above.

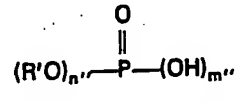
(10) Phosphate ester surfactants:

No. 1 Acid alkyl (or alkenyl) phosphates:



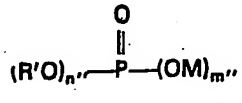
wherein R′ represents an alkyl or alkenyl group having 8—24 carbon atoms, n′+m′ represents 3 and n′ represents a number of 1—2.

No. 2 Alkyl (or alkenyl) phosphates:



wherein R′ has the same meaning as above, n″+m″ represents a number of 3 and n″ represents a number of 1—3.

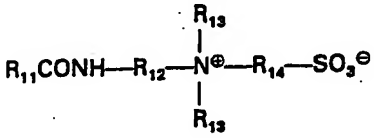
No. 3 Alkyl (or alkenyl) phosphate salts:



wherein R′, n″ and m″ have the same meaning as above and M represents Na, K or Ca.

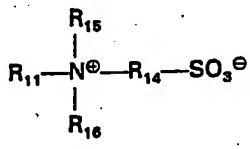
(11) Sulfonic acid-type amphoteric surfactants of the general formulae:

No. 1



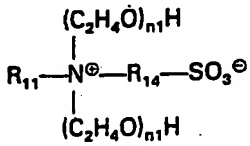
wherein R_{11} represents an alkyl or alkenyl group having 8—24 carbon atoms, R_{12} represents an alkylene group having 1—4 carbon atoms, R_{13} represents an alkyl group having 1—5 carbon atoms, R_{14} represents an alkylene or hydroxyalkylene group having 1—4 carbon atoms.

No. 2



wherein R_{11} and R_{14} have the same meaning as above and R_{15} and R_{16} each represent an alkyl or alkenyl group having 8—24 or 1—5 carbon atoms.

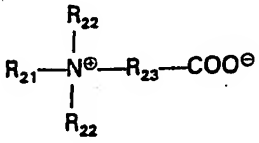
No. 3



wherein R_{11} and R_{14} have the same meaning as above and n1 represents an integer of 1—20.

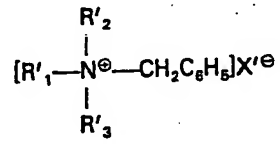
(12) Betaine-type, amphoteric surfactants of the general formulae:

No. 1



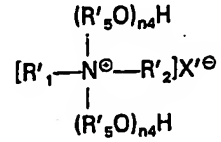
wherein at least one of R'_1, R'_2, R'_3 and R'_4 represents an alkyl or alkenyl group having 8—24 carbon atoms and the remainder represents an alkyl group having 1—5 carbon atoms and X' represents a halogen.

No. 2



wherein R'_1, R'_2, R'_3 and X' have the same meaning as above.

No. 3



wherein R'_1, R'_2 and X' have the same meaning as above, R'_5 represents an alkylene group having 2—3 carbon atoms and n4 represents an integer of 1—20.

The composition preferably contains at least one of the above surfactants in an amount of at least 10 wt.%.

As preferred surfactants, there may be mentioned above surfactants 1), 2), 3), 4), 5), 6), 11)-No. 2, 12)-No. 1, 13), 14), 15), 17) and 18).

[2] Divalent metal ion sequestering agents:

The composition may contain 0—50 wt.% of one or more builder components selected from the group consisting of alkali metal salts or alkanolamine salts of the following compounds:

1) Salts of phosphoric acids such as orthophosphoric acid, pyrophosphoric acid, tripolyphosphoric acid, metaphosphoric acid, hexametaphosphoric acid and phytic acid.

2) Salts of phosphonic acids such as ethane-1,1-disphosphonic acid, ethane,1,1,2-triphosphonic acid, ethane-1-hydroxy-1,1-diphosphonic acid and its derivatives, ethane-hydroxy-1,1,2-triphosphonic acid, ethane-1,2-dicarboxy-1,2-diphosphonic acid and methanehydroxyphosphonic acid.

3) Salts of phosphono carboxylic acids such as 2-phosphonobutane-1,2-dicarboxylic acids, 1-phosphonobutane-2,3,4-tricarboxylic acids and α-methylphosphonosuccinic acid.

4) Salts of amino acids such as aspartic acid, glutamic acid and glycine.

5) Salts of aminopolyacetic acids such as nitrilotriacetic acid, iminodiacetic acid, ethylenediaminetetracetic acid, diethylenetriaminepentaacetic acid, glycol ether diaminetetraacetic acid, hydroxyethyliminodiacetic acid, triethylenetetraminehexaacetic acid and dienkolic acid.

6) High-molecular electrolytes such as polyacrylic acid, polyaconitic acid, polyitaconic acid, polycitractonic acid, polyfumaric acid, polymaleic acid, polymesaconic acid, poly-α-hydroxyacrylic acid, polyvinylphosphonic acid sulphonated polymaleic acid, maleic anhydride/diisobutylene copolymer, maleic anhydride/styrene copolymer, maleic anhydride/methyl vinyl ether copolymer, maleic anhydride/ethylene copolymer, maleic anhydride/ethylene cross-linked copolymer, maleic anhydride/vinyl acetate copolymer, maleic anhydride/acrylonitrile copolymer, maleic anhydride/acrylate ester copolymer, maleic anhydride/butadiene copolymer, maleic anhydride/isoprene copolymer, poly-β-keto carboxylic acid derived from maleic anhydride and carbon monoxide, itaconic acid/ethylene copolymer, itaconic acid/aconitic acid copolymer, itaconic acid/maleic acid copolymer, itaconic acid/acrylic acid copolymer, malonic acid/methylene copolymer, mesaconic acid/fumaric acid copolymer, ethylene glycol/ethylene terephthalate copolymer, vinylpyrrolidone/vinyl acetate copolymer, 1-butene-2,3,4-tricarboxylic acid/itaconic acid/acrylic acid copolymer, quaternary ammonium group-containing polyester polyaldehyde carboxylic acids, cis-isomer of epoxysuccinic acid, poly[N,N-bis(carboxymethyl)acrylamide], poly(hydroxy carboxylic acid), starch succinate, starch maleate, starch terephthalate, starch phosphate ester, dicarboxystarch, dicarboxymethylstarch and cellulose succinate esters.

7) Non-dissociating high-molecular compounds such as polyethylene glycol, polyvinyl alcohol, polyvinylpyrrolidone and cold water-soluble, urethanated polyvinyl alcohol.

8) Salts of dicarboxylic acids such as oxalic acid, malonic acid, succinic acid, glutaric acid, adipic acid, pimelic acid, suberic acid, azelaic acid and decane-1,10-dicarboxylic acid; salts of diglycolic acid, thiodiglycolic acid, oxalacetic acid, hydroxydisuccinic acid, carboxymethylhydroxysuccinic acid and carboxymethyltartronic acid; salts of hydroxy carboxylic acids such as glycolic acid, malic acid, hydroxpivalic acid, tartaric acid, citric acid, lactic acid, gluconic acid, mucic acid, glucuronic acid and dialdehyde starch oxide; salts of itaconic acid, methylsuccinic acid 3-methylglutaric acid, 2,2-dimethylmalonic acid, maleic acid, fumaric acid, glutamic acid, 1,2,3-propanetricarboxylic acid, aconitic acid, 3-butene-1,2,3-tricarboxylic acid, butane,1,2,3,4-tetracarboxylic acid,

[5] Bleaching agents:

A combination of the alkali cellulase of the present invention with a bleaching agent such as sodium percarbonate, sodium perborate, sodium sulfate/hydrogen peroxide adduct or sodium chloride/hydrogen peroxide adduct or/and a photosensitive bleaching dye such as zinc or aluminum salt of sulfonated phthalocyanine further improves the deterging effects.

[6] Enzymes (enzymes which exhibits the essential enzymatic effects thereof in the deterging step):

As the enzymes, the following enzymes may be mentioned (classified with respect to their enzymatic reactivities):

Hydrolases, hydrases, oxido-reductases, desmolases, transferases and isomerases. All of these enzymes may be used in the present invention. Particularly preferred enzymes are hydrolases such as proteases, esterases, carbohydrolases and nucleases.

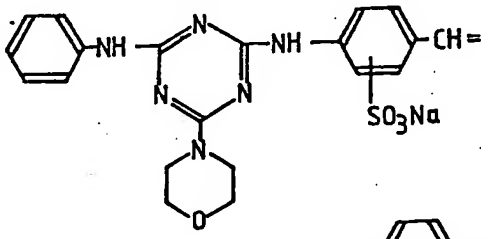
Examples of proteases are pepsin, trypsin, chymotrypsin, collagenase, keratinase, elastase, subtilisin, BPN, papain, bromelin, carboxypeptidases A and B, aminopeptidase and aspergillopeptidases A and B.

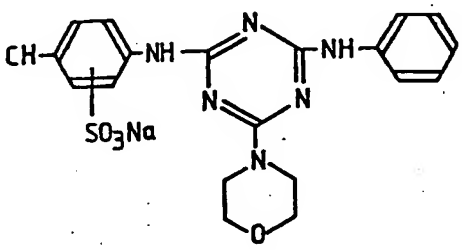
Examples of esterases are gastric lipase, pancreatic lipase, vegetable lipases, phospholipases, cholinesterases and phosphotases.

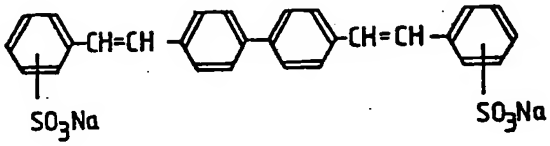
Carbohydrolases other than alkali cellulases include maltase, saccharase, amylase, pectinase, lysozyme, α-glucosidase and β-glucosidase.

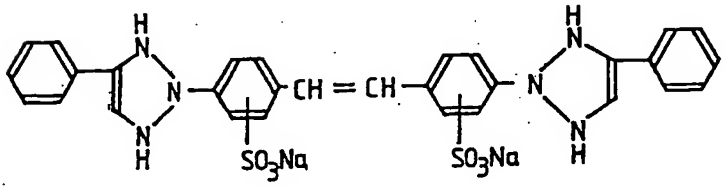
[7] Bluing agents and fluoroescent dyes:

Various bluing agents and fluroescent dyes may be incorporated in the composition, if necessary. For example, compounds of the following structures are recommended:









and bluing agents of the general formulae:

[12] Solubilizers:

The solubilizers include, for example, lower alcohols such as ethanol, benzenesulfonate salts, lower alkylbenzenesulfonate salts such as p-toluenesulfonate salts, glycols such as propylene glycol, acetylbenzenesulfonate salts, acetamides, pyridinedicarboxylic acid amides, benzoate salts and urea.

The following examples will further illustrate the present invention. In the following referential example, the preparation of an alkali cellulase is explained. Unless otherwise state, percentages in the following examples are given by weight.

Referential Example 1

Preparation of alkali cellulase:

Alkali-resistant cellulases according to the present invention are obtained by, for example, a technique disclosed in G. Okada, T. Nishizawa and K. Nishizawa "Biochem. J., *99*, 214 (1966)". More particularly, a crude enzyme solution was extracted from the hepatopancreas of a marine mollusc (Dolabella sp.). The crude enzyme solution was subjected to the starch zone-electrophoresis and carboxymethylcellulose-saccharifying activity of the resulting fraction was measured. The carboxymethylcellulose-saccharifying activity of pH 8.3 was determined from an absorbance (ΔOD) at 660 mμ using an alkaline copper reagent and arsenomolybdate after reacting the fraction with carboxymethylcellulose.

Fraction No.	*Cellulase Activity at pH 8.3 (ΔOD)*
10	0.05
15	0.55
20	0.47
30	0.40
35	0.12

It is understood that fractions Nos. 15, 20 and 30 contain cellulases having a high activity under a weak alkaline condition.

Example 1

Effects of alkali cellulase superior to those of other enzymes on cotton cloths artificially stained with muddy dirts will be shown:

1) Detergent compositions:

	A (%)	*B (%)*
Sodium straight-chain dodecylbenzenesulfonate	10	—
Sodium α-olefinsulfonates (C_{16-18})	5	—
Sodium alkylethoxysulfates (C_{14-15}, $\overline{EO}$=1.5 mols)	2	25
Sodium alkyl sulfates (C_{14-15})	3	—
Soap (beef fatty acid sodium salts)	2	—
Secondary alcohol (C=13.5) ethoxylate ($\overline{EO}$=7)	—	25
Sodium tripolyphosphate	10	—
Crystalline sodium aluminosilicate (type 4A)	10	—
Sodium silicate	10	—
Triethanolamine	—	5
Sodium carbonate	10	—
Potassium carbonate	—	5
Carboxymethylcellulose	1	1
Polyethylene glycol ($\overline{MW}$) 6000)	1	1
Fluorescent dye	0.4	0.3
Bluing agent	—	0.05
Sodium p-toluenesulfonate	2	—
Ethanol	—	8
Water	10	balance
Enzyme	0 or 3	0 or 2
Perfume	0.2	0.1
Glauber's salt	balance	—

Referential Example 2

A culture medium (having a pH value of 10) containing 1.0% of peptone, 1.0% of meat extract, 1.0% of carboxymethyl cellulose (CMC), 0.5% of sodium chloride, 0.1% of potassium dihydrogen phosphate and 1.0% of anhydrous sodium carbonate was inoculated with *Bacillus* N4, a novel species belonging to the genus *Bacillus* (deposited with the FRI deposition number of 1141), separated from the soil collected at Hirosawa, Wako city, Saitama prefecture, and shaking culturing was carried out 37°C for 72 hours. Cells were removed by centrifugal separation to obtain a crude enzyme. The crude enzyme was dried with ethanol according to the customary method to obtain a cellulase powder. Thus, 10 g/l of a cellulase enzyme (having an enzymatic activity of 0.6 unit/mg of the solid at a pH value of 6) (hereinafter referred to as "cellulase N-4") was obtained.

At a pH value of 9, the so-obtained enzyme retained 85% of the activity at a pH value of 6. Incidentally, a commercially available cellulase originating from *Aspergillus niger* had an activity of 0% at a pH value of 9. That is, the cellulase had no activity at a pH value of 9.

Referential Example 3

A flask was charged with 9 ml of a culture medium containing 0.5% of ammonium sulfate, 1.5% of pulp block, 0.02% of glucose, 0.1% of yeast extract, 0.02% of $MgSO_4 \cdot 7H_2O$ and 0.2% of K_2HPO_4, and the culture medium was sterilized at 120°C for 20 minutes. The sterilized culture medium was cooled and mixed with 10 ml of 0.7% aqueous solution of $NaHCO_3$ separately sterilized. The culture medium was then inoculated with a cellulase 212-producing species belonging to the genus *Aeromonas* (deposited with the FRI deposition number of 2306), and shaking culturing was carried out at 37°C for 72 hours. Cells were removed by centrifugal separation to obtain a crude enzyme liquid of cellulase 212. The crude enzyme liquid was dried with ethanol according to the customary procedure to obtain a cellulase powder having an enzymatic activity of 0.55 unit/mg of the solid at a pH value of 6 (hereinafter referred to as "cellulase 212"). At a pH value of 9, the so-obtained enzyme retained 70% of the enzymatic activity at a pH value of 6.

Enzymes used in Examples 2 to 7 are listed as follows.

(1) Cellulase N4
(2) Cellulase 212
(3) Cellulase (supplied by Sigma Co., originating from *Aspergillus niger*, 1.35 units/mg)
(4) Lipase (supplied by Gist Brochades NV, originating from *R. oryzae*)
(5) Amylase (Termamil 60G supplied by Novo Industries Co.)
(6) Protease (Alkalase 2.0M supplied by Novo Industries Co.)

Example 2

A highly alkaline powdery detergent for clothing was prepared according to the following recipe. The pH value of a 0.133% aqueous solution of the detergent was 11.2.

Sodium linear-dodecylbenzene-sulfonate	20% by weight
Soap (sodium salt of beef tallow fatty acid)	2% by weight
Sodium orthophosphate	20% by weight
Sodium metaphosphate	10% by weight
Sodium carbonate	15% by weight
Carboxymethyl cellulose	1% by weight
Polyethylene glycol	1% by weight
Fluorescent dye	0.4% by weight
Glauber salt	balance
Enzyme	0 or 2% by weight
Water	5% by weight

The results of the washing test made on so-prepared detergents are shown in Table 2. Incidentally, in Table 2 and Tables given hereinafter, each detergent is identified by example number-enzyme number (the enzyme-free detergent is identified by example number—(0)).

Table 2

Detergent	*Washing Power Index*
1—(0) (reference detergent)	100
1—(1) (present invention)	103.5
1—(2) (present invention)	104.0
1—(3)	101
1—(4)	100
1—(5)	100
1—(6)	100.5

Carboxymethyl cellulose	1.5% by weight
Polyethylene glycol	1.5% by weight
Fluorescent dye	0.5% by weight
Glauber salt	balance
Water	5% by weight

The results of the washing test are shown in Table 5.

Table 5

Builder	*Enzyme*	*Washing Power Index*
sodium tripolyphosphate, 20%	—	100 (reference detergent)
sodium citrate, 20%	—	98
zeolite type 4A, 20%	—	98.5
sodium citrate, 15%	(6), 5%	98.5
zeolite type 4A, 15%	(6), 5%	98.5
sodium citrate, 15%	(1), 5%	102 (present invention)
sodium citrate, 15%	(2), 5%	102.5 (present invention)
zeolite type 4A, 15%	(1), 5%	101.5 (present invention)
zeolite type 4A, 15%	(2), 5%	102 (present invention)

Example 6

Detergents were prepared according to the recipe adopted in Example 3 by using combinations of enzymes. The results of the washing test made on these detergents are shown in Table 6.

Table 6

Detergent	*Combination of Enzymes (Right Number Indicates % of Enzyme)*	*Washing Power Index*
2—(2) (reference detergent)	(2)=2	100
2—(2)/(4) (present invention	(2)/(4)=1/1	100.5
2—(2)/(5) (present invention	(2)/(5)=1/1	100.5
2—(2)/(6) (present invention)	(2)/(6)=1/1	101
2—(2)/(4)/(6) (present invention)	(2)/(4)/(6)=2/1/1	101.5
2—(2)/(5)/(6) (present invention	(2)/(5)/(6)=2/1/1	101.5
2—(4)/(5)/(6)	(4)/(5)/(6)=2/1/1	98

Example 7

A weakly alkaline powdery detergent for clothing was prepared according to the following recipe.

Sodium alkyl-sulfate ($\overline{C}$=14.5)	15% by weight
Sodium alkylethoxy-sulfate ($\overline{C}$=14.5, $\overline{EO}$=3)	5% by weight
Soap (beef tallow type)	2% by weight
Sodium pyrophosphate	18% by weight
Sodium silicate	13% by weight
Sodium carbonate	5% by weight
Polyethylene glycol	2% by weight
Fluorescent dye	0.2% by weight
Glauber salt	balance
Magnesium silicate	1% by weight
Water	5% by weight
Enzyme	2% by weight
Sodium percarbonate	15% by weight

The results of the washing test made on so-prepared detergents are shown in Table 7.

Table 7

Detergent	*Enzyme*	*Washing Power Index*
6—(6) (reference detergent)	(6)	100
6—(1) (present invention)	(1)	102.5
6—(2) (present invention)	(2)	103